

EFFECTIVE CONTROL OF INVASIVE APPLE SNAIL
(*POMACEA CANALICULATA* LAMARCK) USING
PAECILOMYCES LILACINUS (THOM) SAMSON

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INTRODUCTION

Apple snails of the genus *Pomacea* (Ampul-lariidae), a large, freshwater snail native to South America, were illegally introduced into Thailand to clean fish aquaria because of their ability to consume several kinds of aquatic plants and algae. The apple snail was first discovered in the wild in 1984 (Keawjam & Upatham, 1990). *Pomacea* in Thailand have been identified as *Pomacea canaliculata*, *Pomacea insularum*, and *Pomacea* sp. (Keawjam & Upatham, 1990), but Thaewnon-ngiw et al. (2004) indicated that the gene pool of *Pomacea* in Thailand is panmictic and should be recognized as a single species, *P. canaliculata*. The apple snails were discarded into waterways and rapidly spread out due to a few natural enemies, short maturation time, and potential to lay large numbers of eggs (Cowie, 2002). It has been reported to have caused serious damage to rice crops since 1996, and it is now regarded as being the most important rice pest in Thailand (Janyapeth & Archawakom, 1999; Chanyapate, 2004).

In Thailand molluscicides and insecticides have been used to control the apple snail, as in Taiwan, Japan, and the Philippines, without much success (Mochida, 1991). Some of these compounds have been shown to be extremely toxic to such non-target organisms as fish and other beneficial animals, and they present health risks to workers in the field (Anderson, 1993; Naylor, 1996). Niclosamide, endosulfan, camellia seed cake (residue), and copper sulfate, which are often used to control apple snail in Asian countries, cannot be registered in Japan because of their negative effects on the environment (Wada, 2004). Some botanical extracts have been tested, for instance, root of rotenone (*Derris elliptica*; Kardinan & Iskandar, 1997), but they are not

as effective as saponin. However, all are highly toxic to fish (Suharto, 2000). Wu et al. (2005) reported on the successful use of morpholine, a chemical used as a waxing solvent on fruits to inhibit egg hatching. Recently, vulgarone B, a sesquiterpene from the plant *Artemisia douglasiana*, has reported molluscicidal activity against apple snail (Joshi et al., 2005). Laboratory bioassays indicated that vulgarone B had activity comparable with that of the commercial synthetic molluscicide metaldehyde. Conventional management practices, such as hand picking, fish (Halwart et al., 1996; Teo, 2006), and ducks (Teo, 2001) are labor intensive, uneconomical, and unsustainable. For apple snail management, it is, therefore, imperative to develop an effective and practical method that is not harmful to human health and is environmentally friendly. An effective bacterial biological control agent has been reported by Chobchuenchom & Bhumiratana (2003). However, there are no published data on the use of fungi as a biological control agent for apple snails, whereas there are many publications reported in controlling insects (Sztejnberg et al., 2004; Fiedler & Sosnowska, 2007; Maketon et al., 2007, 2008; Steinkraus et al., 2002) and nematodes (Khan et al., 2004).

In this study, three entomopathogenic fungi and eight nematophagous fungi were screened for ovicidal activity on apple snail egg masses and molluscicidal activity on newly hatched juveniles. The lethal concentration (LC₅₀) and time (LT₅₀) and enzymes production were evaluated for the most virulent fungus based on the hypothesis of enzymes hydrolysis assisting in fungal mycelial penetration through the egg and juvenile cell walls. Furthermore, it was subsequently formed into a wettable powder and tested for its efficacy as a microbial molluscicide in a greenhouse for understanding the full ramifications of such applications.

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MATERIAL AND METHODS

Isolation and Preparation of Conidial Suspension

Nematophagous fungi were isolated from a total of 60 soil samples obtained from various agricultural areas in Thailand. The isolation procedure was initiated by measuring 10 g soil mixed with 100 ml of sterile water in a beaker, with the addition of ten one-day-old apple snails. The number of snail deaths was observed daily for seven days. Death was determined by the lack of response when pricking the snails' head or foot. All dead snails were removed from the beakers, soaked in 10% sodium hypochlorite (Sigma-Aldrich, Missouri) for 5 min, and placed in a Petri dish containing Martin's Streptomycin Medium (Difco, Becton-Dickson, Sparks, Maryland). Fungi isolated were sent to Thailand Institute of Scientific and Technological Research (TISTR) for identification. In addition, three entomopathogenic fungi from our laboratory were used; they were *Metarhizium flavoviride* CKM-083 (Maketon et al., 2007), *Beauveria bassiana* CKB-048 (Maketon & Pitiporntapin, 2006), and *M. anisopliae* CKM-051 (Maketon et al., 2007).

All fungal isolates were grown on potato dextrose agar (Difco, Becton-Dickson, Sparks, Maryland) at $27 \pm 1^\circ\text{C}$ with 75–85% relative humidity in the room. The incubation period was 10–15 days. Conidial (spore) suspensions were made by lightly scraping the fungal culture surface with a sterile cell spreader into a sterile glass container. The conidial clumps were suspended in distilled water with 0.01% Tween 80 (ICI Americas, Norwich, New York). The suspensions were vortexed for 5 min to dissociate clumps and filtered through one layer of cheesecloth to remove conidial clumps and mycelial debris. Concentration of each suspension was diluted to 2×10^8 conidia/ml determined by a Neubauer hemocytometer under a phase-contrast microscope. The suspensions were used on the same day or the day after preparation and shaken before use. The pure fungal culture of the most virulent strain was deposited at TISTR.

Collection and Preparation of Apple Snails

All apple snails used in the laboratory experiment were collected using stick traps to obtain egg clutches around the pond at Kasetsart

University, Bangkok. For ovicidal bioassay, egg clutches (one day after laying) were divided into 1 g each (approximately 200 eggs) using a brush pen, and placed on 9 cm diameter filter paper in a sterile Petri dish. For molluscidal bioassay, the egg clutches were placed above the waterline at room temperature ($27 \pm 1^\circ\text{C}$) until the young snails hatched and fell into the water. The water used for snail rearing was regular tap water left to stand for 24 h before use. Young snails were fed with rice leaves. In each test, ten snails of the same age group were sorted by size and placed into 9 cm diameter filter paper moistened with 1 ml sterile water in a sterile Petri dish with a piece of rice leaf and replaced daily.

Experiment 1 – Fungal Virulence One Day after Egg Laying

Conidial suspensions of eleven fungi (Table 1) were sprayed on one day after laying apple snail eggs using a Thin Layer Chromatography (TLC) sprayer (Merck GmbH, Germany) at concentrations of 1 ml per gram of egg, resulting in approximately 1×10^8 conidia per gram of egg. Thirteen treatments with three replicates, including a water-treated and an untreated control, were performed. The percentage of unhatched apple snail eggs was recorded daily for 14 days after inoculation.

Experiment 2 – Two Most Virulent Fungi One, Five and Seven Days after Egg Laying

Conidial suspensions of the two most effective fungi from Experiment 1 (*Paecilomyces lilacinus* CKP-012 and *P. lilacinus* CKP-032) were prepared at the concentrations of 1×10^8 conidia/ml and then sprayed on one, five, and seven days after laying eggs using a TLC sprayer. Four treatments with five replicates, including a water-treated and an untreated control were performed. The percentage of unhatched eggs was recorded daily for 14 days after inoculation.

Experiment 3 – Two Most Virulent Fungi on One-, Five-, and Ten-Day-Old Juveniles

The experiment was performed exactly the same as Experiment 2, except with test subjects were one-, five-, and ten-day-old juveniles.

Experiment 4 – Lethal Concentration (LC₅₀) and Lethal Time (LT₅₀)

For LC₅₀, conidial suspensions of the most virulent fungus from Experiment 1 were prepared at concentrations ranging from 1x10⁶ to 1x10⁹ conidia/ml and tested on one and five days after laying eggs, and one and five-day-old juveniles. Mortality was checked daily for five days. Similar procedures were followed for LT₅₀, except only one conidial suspension concentration of 1x10⁸ conidia/ml was sprayed on eggs and juveniles. Probit analysis (Finney, 1971) was used for calculation with 95% confidence intervals.

Experiment 5 – Enzymes Produced by the Most Virulent Fungus

Methods of Khan et al. (2003) and Dackman et al. (1989) were employed for protease determination. Skimmed milk (0.2% W/V) was added to minimal medium (Bonants et al., 1995) cultures in 1-liter conical flasks containing 250 ml culture medium and inoculated with the most virulent fungus from Experiment 1 at 1.0x10⁶ conidia/ml. Cultures were incubated on a shaker at 125 rpm for seven days at 27°C. Samples were drawn daily and diluted in a carbonate-bicarbonate buffer pH 10.2 (Dawson et al., 1986). One milliliter of diluted sample was incubated with 1 ml of 1% azocasein at 50°C for 30 min. The reaction was terminated by the addition of 1.5 ml 5% trichloroacetic acid. Non-digested azocasein was separated by centrifuging at 600 x g for 20 min and culture supernatants filtered through 0.2 µm membranes. Protease activity was measured at 345 nm and expressed as dry weight (mg/ml) of azocasein solubilized obtained from the formula $A_{345} \times 0.566 \times \text{dilution factor}$ as described (adapted from Khan et al., 2003).

For chitinase production, minimal medium was supplemented with 0.5% (W/V) chitin. One-liter conical flasks containing 250 ml of medium were inoculated with 1.0x10⁶ conidia/ml of the most virulent fungus and incubated at 125 rpm for 7 days at 27°C. Similar to protease detection, 1.5 ml of sample were drawn daily and incubated with 1 ml of 1% colloidal chitin at 37°C for 2 h (adapted from Dackman et al., 1989; Khan et al., 2003). Precipitate was separated, and the supernatant was measured at 285 nm. Chitinase activity was indicated by the increase in absorbance of N-acetyl-L-glucosamine.

The method of Miller (1959) was modified for measuring the amount of glucose released after an amylase enzyme hydrolysis using starch as a substrate. The reagents used were 1% 3,5-dinitrosalicylic acid, 30% potassium sodium tartrate (Rochelle salt), 0.05% sodium sulfite, and 1% sodium hydroxide. The substrate, reagents, and mycelial-free supernatant mixture were incubated at 37°C for 10 min, and then the change in absorbance at 575 nm was measured.

Lipase activity was estimated following Lee et al. (1999) using a spectrophotometric assay with p-nitrophenyl butyrate as a substrate, which was dissolved in acetonitrile at a concentration of 10 mM. Subsequently, ethanol and 50 mM potassium phosphate buffer (pH 7.5) were added to a final composition of 1:4:95 (v/v/v) of acetonitrile/ethanol/buffer, respectively. The mycelial-free supernatant (0.3 ml) was added to the substrate solution (0.9 ml), and the mixture was incubated at 60°C. After 15 min, enzyme activity was measured by monitoring the change in absorbance at 450 nm, which represents the amount of released p-nitrophenol. All chemicals used were from Sigma-Aldrich, Missouri.

Experiment 6 – Greenhouse Tests

Preparation of Apple Snail Eggs on Rice Stalks: Fifty pairs of adult apple snails were collected from the campus ponds of Kasetsart University, Bangkok, and placed in 20 round containers (50 cm diameter, 30 cm height) with planted rice stalks (strain Tai Chung Native 1) in a greenhouse, where they laid their eggs, two egg masses per container were used.

Preparation of Conidia into a Powder Form: The two most effective fungi from Experiment 1 were suspended in a 3% Tween 80® wetting agent at a final concentration of 1x10⁹ conidia/g, and freeze dried using sterile attapulgit clay (AGSORB-325® LVM-GA, OIL-DRI Corp., IL) as a filler in a Heto FD3 (Denmark) freeze dryer.

Two Best Fungi Compare to Chemicals on Apple Snail Eggs: A chemical molluscicide, niclosamide 70% WP (Bayluscide™, Bayer Cropsciences, Germany) was used as a comparison. Conidial suspensions from both fungal powders were prepared and sprayed on eggs which resulted in approximately 3.33x10⁷

TABLE 1. Percent hatched of one-day-old apple snail eggs after 14 days. Means followed by the same letter in the same column showed no significant difference at 95% by DMRT.

Fungal isolate	Percent hatched		
	7 days after inoculation	10 days after inoculation	14 days after inoculation
<i>Beauveria bassiana</i> CKB-048	76.35 ± 6.70 ^{ab}	85.50 ± 4.16 ^a	90.05 ± 8.77 ^a
<i>Metarhizium anisopliae</i> CKM-051	72.42 ± 7.24 ^b	78.83 ± 6.14 ^b	83.57 ± 6.27 ^b
<i>M. flavoviride</i> CKM-083	80.06 ± 2.13 ^a	86.70 ± 5.65 ^a	87.77 ± 7.89 ^{ab}
<i>Paecilomyces lilacinus</i> CKP-012	8.53 ± 2.21 ^e	10.50 ± 4.10 ^f	12.22 ± 8.37 ^g
<i>P. lilacinus</i> CKP-032	10.90 ± 4.63 ^e	16.23 ± 5.05 ^f	20.43 ± 6.63 ^f
<i>P. lilacinus</i> CKP-073	26.23 ± 8.16 ^c	39.94 ± 6.74 ^d	49.22 ± 8.23 ^d
<i>P. lilacinus</i> CKP-070	25.40 ± 3.13 ^c	40.00 ± 1.25 ^d	46.00 ± 6.80 ^d
<i>P. lilacinus</i> CKP-063	20.75 ± 8.27 ^d	29.53 ± 8.26 ^e	34.15 ± 6.86 ^e
<i>P. lilacinus</i> CKP-083	22.24 ± 7.14 ^d	32.20 ± 2.76 ^{de}	36.47 ± 6.76 ^e
<i>P. lilacinus</i> CKP-093	12.60 ± 2.40 ^e	19.60 ± 8.41 ^f	23.60 ± 7.15 ^f
<i>P. lilacinus</i> CKP-004	30.40 ± 6.76 ^c	52.86 ± 7.10 ^c	63.40 ± 7.24 ^c
Control (untreated)	92.20 ± 4.10 ^a	88.18 ± 6.40 ^a	95.25 ± 3.20 ^a
Control (water treated)	91.80 ± 6.70 ^a	90.10 ± 5.44 ^a	91.04 ± 4.60 ^a
% C.V.	72.03	56.53	51.54

conidia per g egg. Concentration of the known and commercially available molluscicide was 3.50 mg a.i. of niclosamide 70% WP and was sprayed on each egg mass. Four plastic boards 60×60 cm in size were used to prevent aerosol migration at each container while spraying.

Experiment 7 – Effect of Clay and Wetting Agent to One-Day-Old Eggs and Juveniles

The effects of clay and wetting agents used as fillers from Experiment 6 were tested. The sample was prepared as in Experiment 6, except no fungal spore was added, and water was sprayed for a comparison. The percent of one-day-old eggs hatched and the percent of mortality of one-day-old juveniles was recorded daily for 14 days after sprayed.

Statistical Analysis

Duncan’s new multiple range test (DMRT) was used for calculation and interpretation of statistical difference among treatments.

RESULTS

Eleven fungal isolates exhibited larvicidal capability and were identified according to stan-

dard mycological characterization (Domsch et al., 1993) and polymerase chain reaction techniques (Inglis et al., 2005), but there were only two isolates that showed promising results; they were *Paecilomyces lilacinus*: CKP-012 and CKP-032.

Experiment 1 – Fungal Virulence One Day after Egg Laying

The apple snail eggs hatched 7–12 days after deposition. The eggs in the water treated and untreated control treatments started hatching seven days after deposition with 91.04 ± 4.6 and 95.25 ± 3.2% of success (Table 1) (means ± SE). The eggs treated with doses of *M. flavoviride* CKM-083, *B. bassiana* CKB-048, and *M. anisopliae* CKM-051 showed no difference in rates of hatching within the same time frame, and these three isolates were considered ineffective strains. Comparatively, treatments with all eight strains of *P. lilacinus* resulted in delayed egg hatching. Egg hatching percentages at 10 days after inoculation showed similar results to the seven days post-inoculation results. For all eggs infected by isolates of *P. lilacinus* again appeared to have low hatching percentages; the highest hatching rate was still found in CKP-004, followed with CKP-070, CKP-073, CKP-083, CKP-063, CKP-093, CKP-

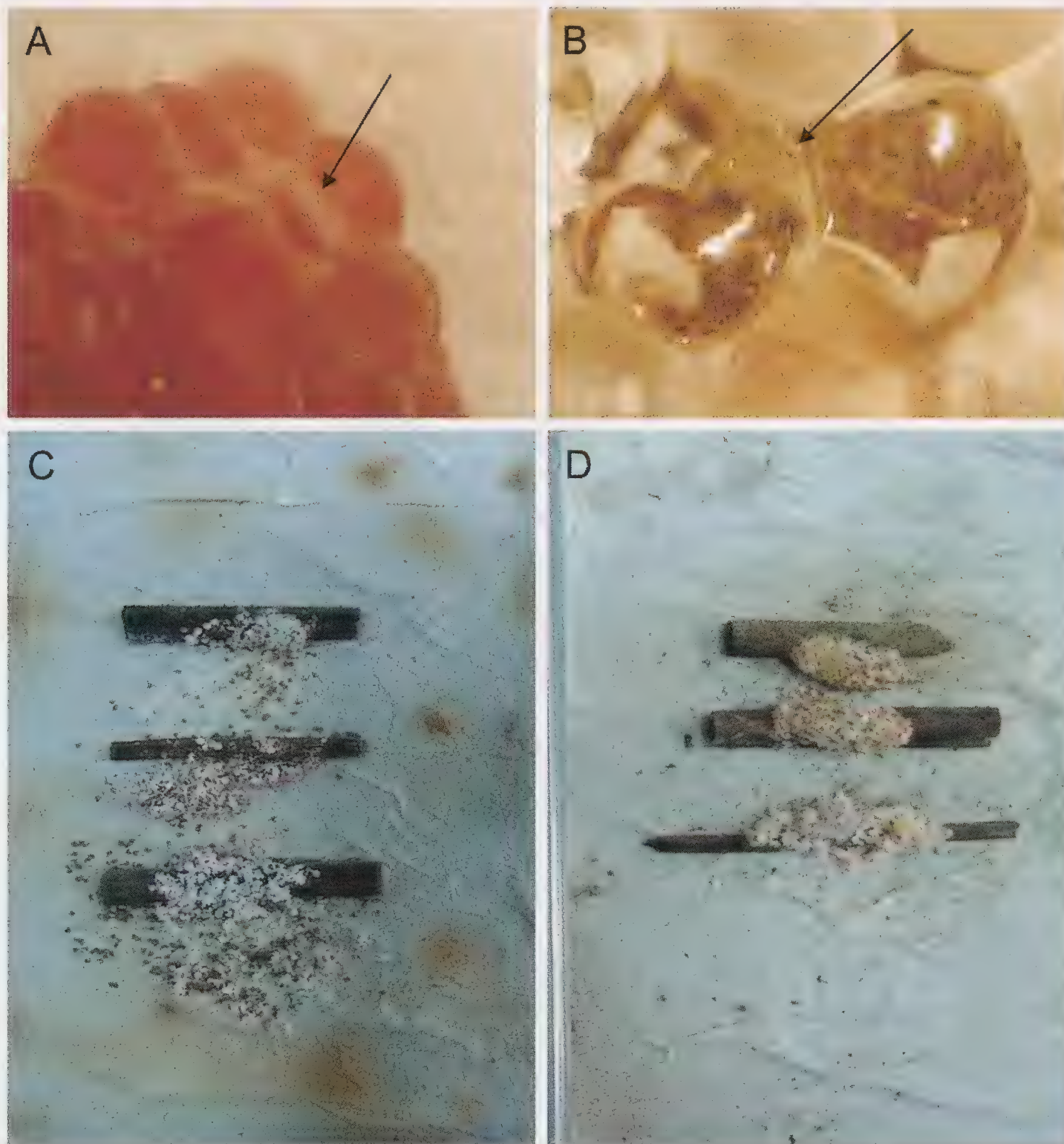


FIG. 1. Apple snail eggs infected by *Paecilomyces lilacinus* CKP-012 showing fungal mycelium development after 4 days (A) and some eggs shell were hydrolyzed after 10 days (B), and most eggs become juveniles from the water treated control after 14 days (C) while only a few hatched from *P. lilacinus* CKP-012 treated after the same period (D).

032, and CKP-012, respectively. Mycelium of CKP-012 appeared on eggs after four days, and some egg shells were hydrolyzed after ten days (Fig. 1).

At 14 days after inoculation, similar results were observed for the high mortality of developing juveniles from the two most effective *P. lilacinus* which were CKP-012 followed by CKP-032.

Experiment 2 – Two Most Virulent Fungi One, Five and Seven Days after Egg Laying

After having screened for the most successive ovicidal behavior, two *P. lilacinus* isolates, CKP-012 and CKP-032, were further studied for their efficacies in controlling different developmental egg stages. After 14 days, CKP-012 was superior to CKP-032. The percent hatched

were 13.67 ± 3.43 , 17.57 ± 4.16 , and 20.55 ± 6.76 for CKP-012 treated at one, five, and seven-day-old eggs, respectively. Also, 19.12 ± 5.61 , 24.17 ± 3.12 , and $31.22 \pm 2.11\%$ hatched from CKP-032 treated at one, five, and seven-day-old eggs, respectively (Fig. 2A). While in the water treated and untreated controls, most eggs become juveniles with only 3 to 6% of the juveniles dying.

Experiment 3 – Two Most Virulent Fungi on One-, Five-, and Ten-Day-Old Juveniles

After two weeks, *P. lilacinus* CKP-012 had a high capability of killing newly hatched juveniles; however, with increasing maturity, juveniles became more tolerant to the fatal effects of the fungi. *P. lilacinus* CKP-012, which resulted in higher mortality than CKP-032. The

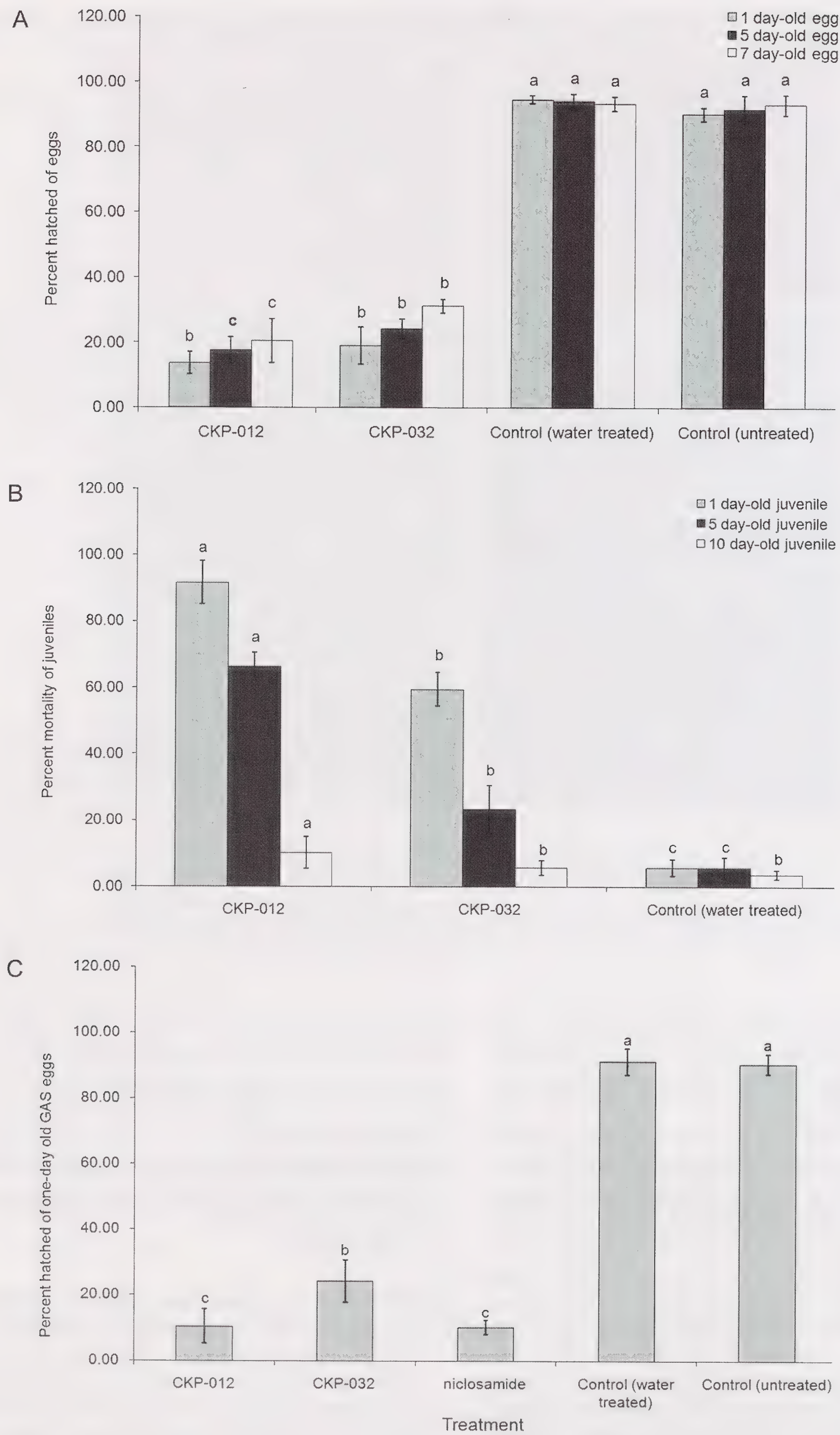


FIG. 2. Percent hatched of apple snail eggs after 14 days. (A) Percent mortality of apple snail juveniles after 14 days; (B) and Percent hatched of one-day-old apple snail eggs after 14 days; (C) Means followed by the same alphabet on the same day showed no significant difference at 95% by DMRT.

TABLE 2. Enzymes production from *Paecilomyces lilacinus* CKP-012.

Day	Protease activity ^a	Chitinase activity ^b	Amylase activity ^c	Lipase activity ^d
1	0.0000	0.0779	0.3140	0.1010
2	0.0000	0.0808	0.3080	0.1040
3	1.6298	0.0869	0.3370	0.0980
4	1.1507	0.0989	0.2970	0.1090
5	1.4787	0.0966	0.1090	0.1610
6	2.7219	0.0954	0.0920	0.2800
7	2.7318	0.1000	0.0950	0.2870
Control	0.0000	0.0000	0.0000	0.0000

^a Protease activity was measured as dry weight (mg/ml) of azocasein solubilized = $A_{345} \times 0.566 \times$ dilution of the sample. Protease and azocasein mixture was incubated at 50°C and pH 10.2 for 30 min.
^b Chitinase activity was measured by the increase in absorbance of N-acetyl-L-glucosamine at 285 nm. Chitinase and chitin mixture was incubated at 37°C and pH 5.1 for 2 h.
^c Amylase activity was measured as 1 μ mole of D-Glucose released from starch solution per min at 37°C for 10 min.
^d Lipase activity was measured as 1 μ mole of p-nitrophenol released from p-nitrophenyl butyrate per min at 60°C for 15 min.

percentages of mortality for one-, five-, and ten-day-old juveniles treated with CKP-012 were 91.67 ± 6.50 , 66.32 ± 4.21 , and 10.20 ± 4.73 , respectively; while those treated with CKP-032 were 59.50 ± 5.10 , 23.30 ± 7.30 , and $5.70 \pm 2.14\%$, respectively (Fig. 2B). Dehydration in the untreated control detrimentally affected the test because young juveniles started dying after four days and were decimated after nine days.

Experiment 4 – Lethal Concentration (LC₅₀) and Lethal Time (LT₅₀)

The level of lethal concentration required for *P. lilacinus* CKP-012 in preventing hatching of 50% apple snail eggs was 8.63×10^7 conidia/ml for one-day-old eggs and 1.58×10^8 conidia/ml for five-day-old eggs. While the LC₅₀ for killing one-day-old and five-day-old juveniles were 1.74×10^8 and 3.71×10^8 conidia/ml respectively. The lethal time required to inhibit the hatching of 50% of the eggs treated after one day was 2.26 days and 4.36 days for those treated on day five. However, longer times were required for killing juveniles at 3.43 days and 4.38 days for one-day-old and five-day-old juveniles respectively.

Experiment 5 – Enzymes Produced by the Most Virulent Fungus

Quantitative study showed that *P. lilacinus* CKP-012 produced four types of enzymes; they are a protein, a chitin, a carbohydrate,

and a lipid digesting enzymes (Table 2). The concentration of four digesting enzymes based on a seven-day trial. The results show that protease, chitinase, and lipase activities tend to increase significantly from day one to day seven. Protease activity increased from 0.000 to 2.7318, chitinase activity increased from 0.0779 to 0.1000, and lipase activity increased from 0.1010 to 0.2870. However, amylase activity decreased from 0.3140 to 0.0950; this might have resulted from the fact that starch was not an appropriate substrate for the specific amylase produced.

Experiment 6 – Greenhouse Tests

Results of a comparative test of two strains of *P. lilacinus*, CPK-012 and CPK-032, against a known chemical molluscicide (niclosamide) are shown in Figure 2c. After 14 days, there were no significant difference in the hatching percentage of one-day-old eggs treated with CKP-012 and niclosamide 70% WP, which were 10.50 ± 5.12 and 10.27 ± 2.10 , respectively ($p > 0.05$). However, both treatments were significantly more effective than *P. lilacinus* CKP-032, which hatched at $28.22 \pm 6.40\%$ while the water's treated and untreated controls had 91.25 ± 4.10 and $90.50 \pm 3.10\%$ hatched, respectively. Developing juveniles in the greenhouse were difficult to observe because newly hatched juveniles immediately migrated into the water and were inaccessible. However, a repellent effect from fungal application was noted because a number of newly introduced female apple snails

would not lay their eggs on previously treated rice stalks. New rice stalks had to be replaced before egg laying could be initiated.

Experiment 7 – Effect of Clay and Wetting Agent to One-Day-Old Eggs and Juveniles

Clay and wetting agents used as fillers had negligible effects on one-day-old eggs, with the percent hatching of 88.86 ± 1.93 and 89.26 ± 2.64 that occurred from water-treated and filler treated, respectively. A similar trend happened in juveniles; the mortality was quite low with 10.00 ± 0.72 and 11.67 ± 0.84 occurring in water-treated and filler treated specimens, respectively.

DISCUSSION

Samson (1974) classified *Paecilomyces lilacinus* as a fungus used for controlling some kind of nematodes that cause plant diseases (Jatala, 1986). Those nematodes mainly belong to the genus *Meloidogyne*, which causes root-knot symptoms in some agricultural plants. Nematophagous fungi penetrate the host cell by means of mechanical pressure as well as enzymatic digestion (Dackman et al., 1989; Morgan et al., 1983; Stirling, 1991). Bonants et al. (1995) found that *P. lilacinus* (CBS 143.75) can produce protease to alter the embryogenesis of the eggs of *Meloidogyne incognita*, essentially stopping the hatching of juveniles. Results from our work showed that *P. lilacinus* CKP-012 can also grow into apple snail egg by penetration through the egg wall and establishing a mycelium in the egg matrix. The mechanism might have occurred from fungal enzymes' hydrolysis on the egg shells, encouraging the mycelium to penetrate because the egg shells not only consist of calcium, but also such biochemical substances as caroteno-protein complexes (Heras et al., 2007). Therefore, other constituents are amenable for enzyme hydrolysis, especially protease and lipase. From this study, the protease content excreted by the fungus was high enough to lyse the protein matrix on the egg shell. For lipase, even though its content was fairly low, it still had an effect in digesting the lipid portion, in this case the carotenoid moiety. Once the fungal mycelium passes through the shell, its enzymes will continue to play an important role in digesting several biochemical constituents in the perivitelline fluid. According to Heras et al. (1998), biochemical composition at stage I is represented by carbohydrates,

proteins, and lipids with 34.8%, 13.0%, and 1.5% dry weight, respectively; these are the major source of nutrients for the embryo. The carbohydrate moieties were not only monosaccharide, which include mannose and galactose, but also N-acetylglucosamine, a chitin precursor (Heras et al., 2007). Thus, a chitinase enzyme may also play an additional role in digesting or inhibiting a complete ovorubin formation. This effect was similar to that found by Khan et al. (2004), who studied the effect of *P. lilacinus* on eggshell of *M. javanica*, the fungus showed its efficacy in disintegration of vitelline, chitin, and lipid layers of *M. javanica*'s eggs. Vitelline and lipid layers support eggs by providing structural uniformity and permeability characteristics, respectively. Damage to these two layers caused by enzyme treatment enabled other metabolites to permeate the eggs causing changes such as swelling of eggs. Therefore, enzymes may facilitate physical penetration and improve efficiency of infection.

P. lilacinus CKP-012 had a high capability for killing newly hatched juveniles; however, with increasing maturity, higher tolerance appeared to the fatal effects of the fungus. This could result from the fact that the snail's shell becomes thicker and possibly more difficult to penetrate. However, from this study, *P. lilacinus* demonstrated promising results as a biocontrol agent for combating the apple snails, especially when applied to egg stage, *P. lilacinus* performed as effectively as a commercial chemical based molluscicidal agent. Therefore, *P. lilacinus* should be considered as a biological control agent for controlling apple snail in an integrated pest management program.

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